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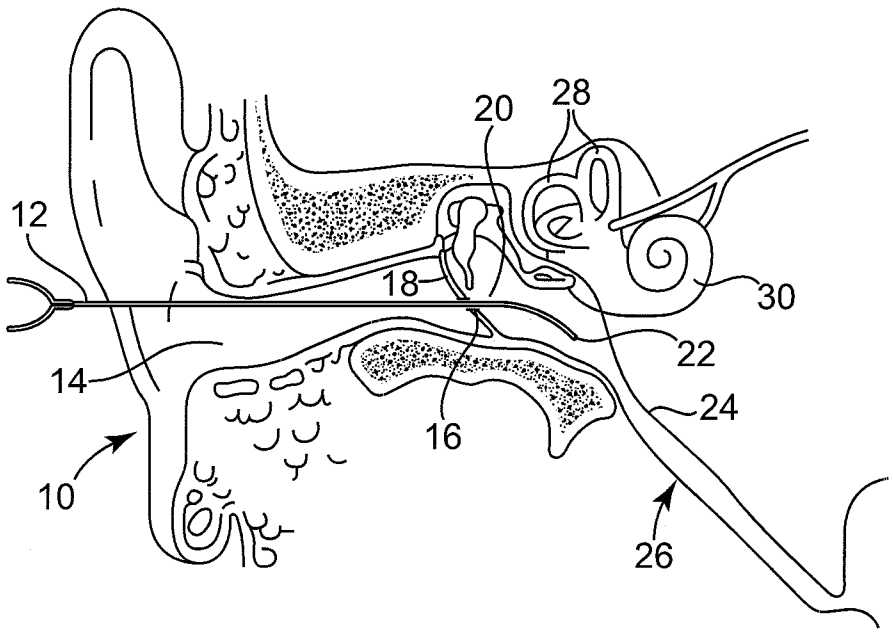
(54) Title: ANTIBACTERIAL EXTRACELLULAR POLYSACCHARIDE SOLVATING SYSTEM



(57) Abstract: Chronic Otitis Media with Effusion (COME), Recurrent Acute Otitis Media (RAOM), cholesteatoma, chronic rhinosinusitis and other bacterial ear, nose or throat conditions may be treated by applying a solvating system to a bacterial biofilm attached or adhered to the treatment site. The solvating system disrupts the biofilm and aids in its removal.

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ANTIBACTERIAL EXTRACELLULAR POLYSACCHARIDE SOLVATING SYSTEM

FIELD OF THE INVENTION

[0001] This invention relates to the treatment of bacterial ear, nose or throat conditions including chronic otitis media with effusion (COME), Recurrent Acute Otitis Media (RAOM), cholesteatoma, and chronic rhinosinusitis.

BACKGROUND

[0002] COME and RAOM are inflammatory diseases of the middle ear. Biofilm formation may be a factor in the pathogenesis of COME, see Post, J.C., "Direct evidence of bacterial biofilms in otitis media", Laryngoscope 111(12):2083-94 (2001), Ehrlich et al., "Mucosal Biofilm Formation on Middle-Ear Mucosa in the Chinchilla Model of Otitis Media", JAMA 287(13):1710-15 (2002) and Fergie, N et al., "Is otitis media with effusion a biofilm infection?", Clin Otolaryngol Allied Sci. 29(1):38-46 (2004). Biofilms form when bacteria interact with a surface to form polymeric films (sometimes referred to as exopolysaccharide or extracellular polysaccharide polymers) that coat the surface and provide a living colony for further bacterial proliferation. Bacteria lodged in biofilms are much more difficult to remove or kill than bacteria in a plaktonic (suspended) state, and are extremely resistant to many antibiotics and biocides. Both the extracellular polysaccharide (EPS) matrix and the toxins produced by a number of different bacteria have been shown to cause inflammation by the host. It appears that the chronic inflammation associated with COME and RAOM is a host response to the bacterial biofilm.

[0003] COME and RAOM are usually initially treated using oral antibiotics and then, if need be, are more aggressively treated by placement of a tympanostomy tube. Occasionally in cases involving severe infection or high mucous content in the middle ear, the middle ear may be irrigated (e.g., with saline solution). While tympanostomy tubes work on most patients, about 20% of patients who undergo primary tympanostomy tube placement require an additional surgery (an adenoidectomy, a second set of tympanostomy

tubes, and usually both an adenoidectomy and tympanostomy tube placement) to treat persistent COME or persistent RAOM.

[0004] Cholesteatoma is another ear disease condition of concern. Although generally thought to be primarily a cyst comprised of dermal cells, bacteria biofilms have also been implicated in this disease, see Chole et al., "Evidence for Biofilm Formation in Cholesteatomas", Arch Otolaryngol Head Neck Surg. 128, pp. 1129-33 (Oct. 2002). In cholesteatoma, bacterial biofilms appear to form, incite inflammation, and cause generation of a benign tumor composed mainly of bacteria at its core and dermal cells. The tumor can erode both the ossicular chain (hearing bones) and the mastoid bone, detrimentally affecting hearing. Surgical exposure and excision is the most common treatment for cholesteatoma removal. Up to 25% of these procedures fail due to recurrence of the cholesteatoma and thus require additional surgery or other treatment.

[0005] Chronic rhinosinusitis (CRS) is inflammation of the paranasal sinuses and is associated with anterior or posterior nasal discharge, nasal obstruction or facial pressure, pain or fullness lasting for at least about twelve weeks. CRS affects an estimated 10% or more of the U.S. population. Most patients with CRS are initially treated with medical therapy, but hundreds of thousands undergo functional endoscopic sinus surgery (FESS) for refractory CRS every year. Patients with CRS often have a reduced quality of life, and may require billions of dollars in annual health-care and lost work time costs. CRS is a Th1 and Th2 inflammatory response to a number of mechanisms including but not limited to bacterial toxins, extracellular polysaccharide matrices secreted by bacteria and contained within a bacterial biofilm, fungi, developed allergic reactions to both bacteria and fungi (IgE) and auto immune disorders,. Bacteria associated with CRS and its accompanying inflammation include *Staphylococcus aureus, Pseudomonas aeruginosa, Streptococcus pneumonia, Haemophilus influenzae* and *Moraxella catarrhalis*. Biofilms containing one or more of these species and possibly also containing fungi may be a factor in the pathogenesis of CRS, see e.g., Ramadan et al., "Chronic rhinosinusitis and biofilms", Otolaryngol Head Neck Surg. 132:414-417 (2005) and Ferguson et al., "Demonstration of Biofilm in Human Bacterial Chronic Rhinosinusitis", Am J Rhinol, 5:19, pp. 452-57 (2005). The extracellular polysaccharide (EPS) matrix, the toxins

produced by the bacterial colony, and the fungi that the bacterial biofilm may harbor may each be capable of inciting an inflammatory response from the host.

[0006] The etiology and chronicity of COME, RAOM, cholesteatoma and CRS appear to be related to the presence of bacterial biofilms as well as their recalcitrance post-surgery.

SUMMARY OF THE INVENTION

[0007] Saline solutions and antibiotics may be applied to bacterial biofilms in the ear nose or throat but in difficult cases may not provide adequate relief from chronic conditions. Various techniques and products have been employed to remove or kill bacteria in biofilms found in dental water lines and on medical instruments or other extracorporeal surfaces, but may be poorly suited for treating human tissues. It would be desirable to remove or kill bacteria inhabiting a biofilms within the ear, nose or throat, and if possible to remove or disrupt the biofilm itself sufficiently to discourage bacterial recolonization and biofilm reformation. It would also be desirable to do so while meeting biocompatibility requirements for contact with human tissue, and while using small dosages of administered materials and short periods of application. It has now been discovered that a solvating system comprising a metal ion sequestering agent and surfactant is surprisingly effective in disrupting bacterial biofilms in the ear, nose or throat while being gentle enough for application directly onto delicate or sensitive tissues.

[0008] The invention provides in one aspect the use of a metal ion sequestering agent and greater than 0.2 wt. % of a surfactant which can detach, remove or otherwise disrupt at least a part of a biofilm attached or adhered to at least a portion of the middle or inner ear, to a surface within a nasal or sinus cavity, or to oral or pharyngeal tissue, to manufacture a medicament for the treatment of bacterial ear, nose or throat conditions.

[0009] The invention provides in another aspect the use of (a) a metal ion sequestering agent, (b) a zwitterionic surfactant which can detach, remove or otherwise disrupt at least a part of a biofilm attached or adhered to at least a portion of the middle or inner ear, to a surface within a nasal or sinus cavity, or to oral or pharyngeal tissue, and (c) sufficient buffer to provide a pH greater than 5, to manufacture a medicament for the treatment of bacterial ear, nose or throat conditions.

[0010] The invention provides in another aspect a method for treating bacterial ear, nose or throat conditions, which method comprises:

a) applying a solvating system comprising a metal ion sequestering agent and greater than 0.2 wt. % surfactant to a bacterial biofilm attached or adhered to at least a portion of the middle or inner ear, to a surface within a nasal or sinus cavity, or to oral or pharyngeal tissue, and

b) detaching, removing or otherwise disrupting at least a part of the biofilm.

[0011] The invention provides in yet another aspect a method for treating bacterial ear, nose or throat conditions, which method comprises:

a) applying a solvating system comprising a metal ion sequestering agent, a zwitterionic surfactant, and sufficient buffer so that the solvating system has a pH greater than 5 to a bacterial biofilm attached or adhered to at least a portion of the middle or inner ear, to a surface within a nasal or sinus cavity, or to oral or pharyngeal tissue, and

b) detaching, removing or otherwise disrupting at least a part of the biofilm.

[0012] The invention provides in yet another aspect a solvating system for disrupting bacterial biofilms on tissue, the composition comprising a metal ion sequestering agent, greater than 0.2 wt. % surfactant, and an antimicrobial agent.

[0013] The invention provides in a further aspect a solvating system for disrupting bacterial biofilms on tissue, the composition comprising a metal ion sequestering agent, a zwitterionic surfactant, and sufficient buffer so that the solvating system has a pH greater than 5.

[0014] The disclosed use, method and system may be used for treatment or post-operative care of the middle or inner ear, and for rhinologic, oral or pharyngic treatment or post-operative care. The disclosed method and system may also be used to treat maladies or chronic conditions including chronic otitis media with effusion, recurrent acute otitis media, cholesteatoma, chronic rhinosinusitis and other bacterial ear, sinus, oral cavity or throat conditions.

BRIEF DESCRIPTION OF THE DRAWING

[0015] **Fig. 1** is a schematic cross-sectional view of a middle ear undergoing treatment via the disclosed method.

[0016] **Fig. 2** is an enlarged view of a portion of **Fig. 1** showing application of the disclosed solvating system to a bacterial biofilm proximate the Eustachian tube isthmus.

[0017] Like reference symbols in the various figures of the drawing indicate like elements. The elements in the drawing are not to scale.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0018] The following detailed description describes certain embodiments and is not to be taken in a limiting sense. All weights, amounts and ratios herein are by weight, unless otherwise specifically noted. The terms shown below have the following meanings:

[0019] The term "antimicrobial agent" refers to a substance having the ability to cause greater than a 90% numeric reduction (*viz.*, at least a 1-log order reduction) in a population of one or more of *Staphylococcus aureus, Pseudomonas aeruginosa, Streptococcus pneumonia, Haemophilus influenzae* or *Moraxella catarrhalis* bacteria using the bacterial plate count procedure described below in the Examples.

[0020] The terms "attached" and "adhered" when used in reference to a bacterial biofilm and a surface mean that the biofilm is established on and at least partially coats or covers the surface, and has some resistance to removal from the surface. As the nature of this relationship is complex and poorly understood, no particular mechanism of attachment or adherence is intended by such usage.

[0021] The term "bacterial biofilm" means a community of bacteria attached to a surface, with the organisms in the community being contained within an extracellular polymeric substance (EPS) matrix produced by the bacteria.

[0022] The term "biocompatible" when used in reference to a substance means that the substance presents no significant deleterious or untoward effects upon the body.

[0023] The term "biodegradable" when used in reference to a substance means that the substance will degrade or erode in vivo to form smaller chemical species. Such degradation process may be enzymatic, chemical or physical.

[0024] The term "bioresorbable" when used in reference to a substance means that the substance is capable of being absorbed by the body.

[0025] The terms "detaching", "removing" and "disrupting" when used in reference to a bacterial biofilm attached or adhered to a surface mean that at least a significant amount of the biofilm initially present on the surface no longer is attached or adhered to the surface. No particular mechanism of detachment, removal or disruption is intended by such usage.

[0026] The term "sequestering agent" means a chemical that will combine with another material, especially a metal ion, to discourage or prevent the material from coming out of solution. The term "metal ion sequestering agent" means a sequestering agent that will combine with one or more metal ions such as alkali metals, alkaline earth metals, iron and the like to discourage or prevent the metal ion from coming out of solution. In order of increasing atomic number the alkali metals are lithium, sodium, potassium, rubidium, cesium, and francium, and the alkaline earth metals are beryllium, magnesium, calcium, strontium, barium, and radium.

[0027] The term "solvating" means to form a solution or dispersion containing a solvent or other carrier within which a solute is dissolved or suspended.

[0028] Referring to **Fig. 1**, one method for applying the solvating system to a treatment site such as within ear **10** may be performed by inserting cannula **12** through ear canal **14** and ear tube **16** (which may for example be placed via myringotomy) in tympanic membrane **18** and thence into middle ear **20**. Cannula **12** may also be inserted in other ways without myringotomy, such as through a needle or other guidance device directed through the ear, Eustachian tubes or nose, and operated blindly or by using guided techniques such as microendoscopy, virtual image guided endoscopy, or image guided surgery using a flexible, tip tracked device. As shown in **Fig. 1**, the distal end **22** of cannula **12** is positioned above isthmus **24** of Eustachian tube **26**. Cannula **12** may be positioned and if need be modified in shape or size so as to treat other portions of middle ear **20** (which for purposes of this discussion will be deemed to include at least the tympanic membrane, the lining of the middle ear, interior structures such as the ossicular chain and bordering structures such as the mastoid), to treat portions of the inner ear (which for purposes of this discussion will be deemed to include at least semicircular

canals **28** and cochlea **30**), or to treat other sites in the sinus cavities, oral cavity or throat. For example, if treatment in the inner ear is desired, a further access opening (e.g., in a membrane near the round window or oval window) may be made.

[0029] **Fig. 2** shows an enlarged view of a portion of **Fig. 1**. The solvating system may be dispensed through orifices **34** located in sidewall **36**, and dripped, sprayed or otherwise administered onto a bacterial biofilm such as biofilm **38** disposed on upper portion **40** of Eustachian tube **26**.

[0030] The disclosed method may be performed in other ear, nose or throat procedures. For example, the method may be performed in the nasal or sinus cavities of a patient. The method may also be performed by applying the solvating system to the tonsils, adenoids or adjacent tissue. This may be done when the tonsils and adenoids are intact, or performed postoperatively after removal of the tonsils, adenoids or both tonsils and adenoids, e.g., by applying the solvating system to the throat, such as to the tonsillar fossa. Further details regarding such procedures are contained in copending application Serial No. (attorney docket no. 151-P-28168WO01), filed even date herewith, the entire disclosure of which is incorporated herein by reference.

[0031] The solvating system can be used to break down bacterial biofilms on ear, nose or throat tissues and consequently aid in their detachment, removal or disruption. The solvating system preferably is biocompatible with the delicate tissues and structures of the middle or inner ear, and desirably does not contain ingredients which might potentially harm such tissues or structures or unduly compromise long-term hearing. The solvating system desirably has a sufficiently low viscosity to enable easy delivery to the bacterial biofilm using for example power spray or other spray application, lavage, misting, mopping, wicking or dripping. The solvating system desirably also may be easily removed from the treatment site by subsequent flushing, rinsing, draining or absorption. While not wishing to be bound by theory, the metal ion sequestering agent may complex, bind or otherwise tie up metal ions which may crosslink, bridge or otherwise assist in binding together the polymer chains in an exopolysaccharide or extracellular polysaccharide matrix. The solvating agent may then surround the unbound polymer chains or fragments, breaking down the matrix, solvating the unbound polymer chains or fragments, and bringing them into solution or suspension where they can be easily flushed

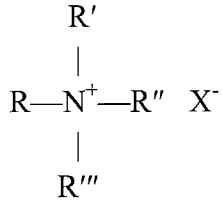
or otherwise removed from the treated middle ear or inner ear tissues or structures using for example additional amounts of the solvating system or a separate rinsing agent.

[0032] The metal ion sequestering agent desirably is a mild acid whose acidity is sufficient to sequester one or more metal ions in the exopolysaccharide or extracellular polysaccharide matrix, but which is not so acidic so as to harm the treated tissue. Metal ions of particular interest (due to their likely involvement in the targeted bacterial biofilms) include sodium, calcium and iron. The metal ion sequestering agent desirably is water-soluble, nontoxic and if used in the ear not prone to aggravate long-term hearing loss. Representative acids include but are not limited to carboxylic acids, diacids, or triacids such as formic acid, acetic acid, chloroacetic acid, dichloroacetic acid, oxalic acid, oxamic acid, glycolic acid, lactic acid, pyruvic acid, aspartic acid, fumaric acid, maleic acid, succinic acid, iminodiacetic acid, glutaric acid, 2-ketoglutaric acid, glutamic acid, adipic acid, citric acid, glucuronic acid, mucic acid, nitrilotriacetic acid, salicylic acid, ketopimelic acid, benzoic acid, mandelic acid, chloromandelic acid, phenylacetic acid, phthalic acid and boric acid; mineral acids such as hydrochloric acid, orthophosphoric acid and phosphonic acid; and mixtures thereof. Citric acid is a preferred acid. The metal ion sequestering agent may for example be present at a concentration of at least about 0.01 M, at least about 0.05 M or at least about 0.1 M, e.g., about 0.01 to about 0.5 M, about 0.05 to about 0.4 M or about 0.1 to about 0.3 M. Increased metal ion sequestering agent amounts may promote faster biofilm breakup.

[0033] The solvating system also includes a surfactant. The surfactant desirably is water-soluble and nontoxic. Exemplary surfactants include anionic surfactants, nonionic surfactants, cationic surfactants and zwitterionic surfactants. Exemplary anionic surfactants include but are not limited to C_6-C_{24} alkylbenzene sulfonates; C_6-C_{24} olefin sulfonates; C_6-C_{24} paraffin sulfonates; cumene sulfonate; xylene sulfonate; C_6-C_{24} alkyl naphthalene sulfonates; C_6-C_{24} alkyl or dialkyl diphenyl ether sulfonates or disulfonates, C_4-C_{24} mono or dialkyl sulfosuccinates; sulfonated or sulfated fatty acids; C_6-C_{24} alcohol sulfates (for example C_6-C_{12} alcohol sulfates); C_6-C_{24} alcohol ether sulfates having 1 to about 20 ethylene oxide groups; C_4-C_{24} alkyl, aryl or alkaryl phosphate esters or their alkoxylated analogues having 1 to about 40 ethylene, propylene or butylene oxide units; and mixtures thereof. For example, the anionic surfactant may be sodium

chenodeoxycholate, N-lauroylsarcosine sodium salt, lithium dodecyl sulfate, 1-octanesulfonic acid sodium salt, sodium cholate hydrate, sodium deoxycholate, sodium dodecyl sulfate (also known as sodium lauryl sulfate) or sodium glycodeoxycholate.

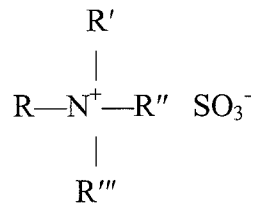
[0034] Exemplary cationic surfactants include but are not limited to quaternary amine compounds having the formula:



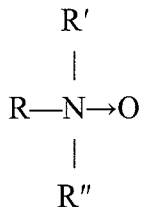
where R, R′, R″ and R‴ are each a C_1-C_{24} alkyl, aryl or aralkyl group that can optionally contain one or more P, O, S or N heteroatoms, and X is F, Cl, Br, I or an alkyl sulfate. For example, the cationic surfactant may be hexadecylpyridinium chloride monohydrate or hexadecyltrimethylammonium bromide.

[0035] Exemplary nonionic surfactants include but are not limited to C_6-C_{24} alcohol ethoxylates (for example C_6-C_{14} alcohol ethoxylates) having 1 to about 20 ethylene oxide groups (for example about 9 to about 20 ethylene oxide groups); C_6-C_{24} alkylphenol ethoxylates (for example C_8-C_{10} alkylphenol ethoxylates) having 1 to about 100 ethylene oxide groups (for example about 12 to about 20 ethylene oxide groups); C_6-C_{24} alkylpolyglycosides (for example C_6-C_{20} alkylpolyglycosides) having 1 to about 20 glycoside groups (for example about 9 to about 20 glycoside groups); C_6-C_{24} fatty acid ester ethoxylates, propoxylates or glycerides; C4-C_{24} mono or di alkanolamides; and mixtures thereof. For example, the nonionic surfactant may be polyoxyethyleneglycol dodecyl ether, N-decanoyl-N-methylglucamine, digitonin, n-dodecyl B-D-maltoside, octyl B-D-glucopyranoside, octylphenol ethoxylate, polyoxyethylene (8) isooctyl phenyl ether, polyoxyethylene sorbitan monolaurate or polyoxyethylene (20) sorbitan monooleate.

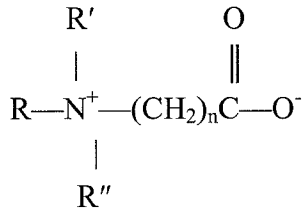
[0036] Exemplary zwitterionic surfactants include but are not limited to aminoalkylsulfonate compounds having the formula:



where R, R′, R″ and R‴ are each a C_1-C_{24} alkyl, aryl or aralkyl group that can optionally contain one or more P, O, S or N heteroatoms; amine oxide compounds having the formula:



where R, R′ and R″ are each a C_1-C_{24} alkyl, aryl or aralkyl group that can optionally contain one or more P, O, S or N heteroatoms; and betaine compounds having the formula:



where R, R′ and R″ are each a C_1-C_{24} alkyl, aryl or aralkyl group that can optionally contain one or more P, O, S or N heteroatoms, and n is about 1 to about 10. For example, the zwitterionic surfactant may be 3-[(3-cholamidopropyl) dimethylammonio]-2-hydroxy-1-propane sulfonate, 3-[(3-cholamidopropyl) dimethylammonio]-1-propane sulfonate (sometimes referred to as CHAPS), 3-(decyldimethylammonio) propanesulfonate inner salt (sometimes referred to as caprylyl sulfobetaine), or N-dodecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate.

[0037] Preferred surfactants include alkyl sulfates, alkyl sulfonates, aryl sulfonates and zwitterionic surfactants. The desired surfactants may be obtained as pure compounds or in some instances may be obtained by using products such as liquid Castile soap. The surfactant may for example be present at a concentration of at least about 0.002 M, at least about 0.005 M or at least about 0.01 M, e.g., about 0.002 to about 1 M, about 0.005 to about 0.7 M or about 0.01 to about 0.5 M. Expressed on a weight basis, the surfactant preferably is greater than 0.2 wt. % of the solvating system and may for example be about

0.3% to about 30%, about 0.5% to about 25% or about 1% to about 20% of the solvating system. Increased surfactant amounts may promote faster biofilm breakup.

[0038] The solvating system may optionally include a variety of other ingredients, including water and other solvents (e.g., alcohols), buffering agents, antimicrobial agents and a variety of adjuvants. Preferably the solvating system contains water and one or more buffering agents. The buffering agent preferably maintains the solvating system at an appropriate pH for contacting human tissue, and desirably at a pH greater than 5. For example, the solvating system may be buffered to have a near-neutral pH, e.g., a pH greater than 5 and less than 8.5. Buffering agents may for example be up to about 25% of the solvating system. Exemplary buffering agents include but are not limited to potassium chloride, glycine, potassium hydrogen phthalate, sodium acetate, potassium hydrogen phthalate, barbitone sodium and sodium citrate. When the metal ion sequestering agent is a mild acid, the buffering agent desirably is a salt of that acid.

[0039] Solvating systems containing one or more antimicrobial agents are also preferred. The EPS matrix allows the biofilm to stick to an underlying surface and also protects the embedded organisms; thus, bacteria in biofilms are approximately 100 to 1000 times more resistant to the effects of antibiotics than planktonic bacteria. After the biofilm has been broken down into unbound polymers or fragments and solvated or otherwise disrupted by the solvating system, an antimicrobial agent can much more effectively attack the remaining bacteria. Exemplary antimicrobial agents include active oxygen compounds such as hydrogen peroxide, isolated or equilibrium derived or isolated peracids such as chloroperbenzoic acids, peracetic acid, perheptanoic acid, peroctanoic acid, perdecanoic acid, performic acid, percitric acid, perglycolic acid, perlactic acid, perbenzoic acid, and monoester peracids derived from diacids or diesters such as adipic, succinic, glutaric, or malonic acid; aminoglycosides; amphenicols; ampicillins; ansamycins; beta-lactams such as carbacephems, carbapenems, cephalosporins, cephamycins, monobactams, oxacephems, penicillins and any of their derivatives; carboxylic esters such as p-hydroxy alkyl benzoates and alkyl cinnamates; chitosan salts; cubic-phase lipids; gallium-containing antimicrobial agents such as gallium acetylacetonate, gallium bromide, gallium chloride, gallium fluoride, gallium iodide, gallium maltolate, gallium nitrate, gallium nitride, gallium percolate, gallium phosphide and gallium sulfate; iodo-compounds and

other active halogen compounds such as iodine, interhalides, polyhalides, metal hypochlorites, hypochlorous acid, metal hypobromites, hypobromous acid, chloro- and bromo-hydantoins, chlorine dioxide and sodium chlorite; lincosamides; macrolides; nitrofurans; organic peroxides including benzoyl peroxide and alkyl benzoyl peroxides; ozone; phenolic derivatives including o-phenyl phenol, o-benzyl-p-chlorophenol, tert-amyl phenol and C_1-C_6 alkyl hydroxy benzoates; quaternary ammonium compounds such as alkyldimethylbenzyl ammonium chloride and dialkyldimethyl ammonium chloride; quinolines; singlet oxygen generators; sulfonamides; sulfones; sulfonic acids such as dodecylbenzene sulfonic acid; tetracycline antibiotics such as tetracycline, chlortetracycline, oxytetracycline, demecocycline, doxycycline, lymecycline, mecloclycline, methacycline, methocycline, minocycline, and the like; vancomycin; derivatives thereof and mixtures thereof. Many of these recited agents represent classes containing useful specific materials whose individual utility will be recognized by persons having ordinary skill in the art. For example, exemplary penicillins include but are not limited to amdinocillin, amdinocillin pivoxil, amoxicillin ampicillin, apalcillin, aspoxicillin, axidocillin, azlocillin, acampicillin, bacampicillin, benzylpenicillinic acid, benzylpenicillin sodium, carbenicillin, carindacillin, clometocillin, cloxacillin, cyclacillin, dicloxacillin, epicillin, fenbenicillin, floxacillin, hetacillin, lenampicillin, metampicillin, methicillin sodium, mezlocillin, nafcillin sodium, oxacillin, penamecillin, penethamate hydriodide, penicillin G benethamine, penicillin G benzathine, penicillin G benzhydrylamine, penicillin G calcium, penicillin G hydrabamine, penicillin G potassium, penicillin G. procaine, penicillin N, penicillin O, penicillin V, penicillin V banzathine, penicillin V hydrabamine, penimepicycline, phenethicillin potassium, piperacillin, pivampicillin propicillin, quinacillin, sulbenicillin, sultamicillin, talampicillin, temocillin, ticarcillin and mixtures thereof or with other materials (e.g., penicillins combined with clavulanic aid such as the combination of amoxicillin and clavulanic acid available as AUGMENTIN™ from GlaxoSmithKline).

[0040] Preferably the antimicrobial agent provides greater than a 99% numeric reduction (*viz.*, at least a 2-log order reduction), greater than a 99.9% numeric reduction (*viz.*, at least a 3-log order reduction), greater than a 99.99% numeric reduction (*viz.*, at least a 4-log order reduction) or greater than a 99.999% numeric reduction (*viz.*, at least a

5-log order reduction) in a population of one or more of *S. aureus, P. aeruginosa, S. pneumonia, H. influenzae* or *M. catarrhalis* bacteria using the bacterial plate count procedure described below in the Examples.

[0041] The solvating system may contain additional therapeutic agents. Exemplary therapeutic agents include any material suitable for otologic, rhinologic or pharyngic use including analgesics, anti-cholinergics, anti-fungal agents, antihistamines, blood products, steroidal or non-steroidal anti-inflammatory agents, anti-parasitic agents, antiviral agents, biostatic compositions, chemotherapeutic/antineoplastic agents, cytokines, decongestants, immunosuppressors, mucolytics, nucleic acids, peptides, proteins, steroids, vasoconstrictors, vitamins, mixtures thereof, and other therapeutic materials that will be apparent to those skilled in the art. Other adjuvants that may be included in the solvating system include dyes, pigments or other colorants (e.g., FD & C Red No. 3, FD & C Red No. 20, FD & C Yellow No. 6, FD & C Blue No.2, D & C Green No. 5, D & C Orange No. 4, D & C Red No. 8, caramel, titanium dioxide, fruit or vegetable colorants such as beet powder or beta-carotene, turmeric, paprika and other materials that will be familiar to those skilled in the art); indicators; flavoring or sweetening agents including but not limited to anise oil, cherry, cinnamon oil, citrus oil (e.g., lemon, lime or orange oil), cocoa, eucalyptus, herbal aromatics (e.g., clove oil, sage oil or cassia oil), lactose, maltose, menthol, peppermint oil, saccharine, sodium cyclamate, spearmint oil, sorbitol, sucrose, vanillin, wintergreen oil, xylitol and mixtures thereof; antioxidants; antifoam agents; and rheology modifiers including thickeners and thixotropes.

[0042] The solvating system desirably is applied in at least an amount and thickness sufficient to cover the desired portion of the biofilm. It may for example be convenient to locate or make a suitable opening near the treatment site (e.g., a myringotomy for some treatments in the middle ear) so that a catheter, cannula, syringe, introducer or other conduit appropriate for delivery of the solvating system may be pushed through the opening. If treatment in the inner ear is desired, a further access opening may likewise be made as noted above. The solvating system may be applied to the targeted tissue and to a targeted biofilm contained therein or thereon so that the biofilm and its organisms are disrupted, solvated and subsequently removed. The treatment may involve chemical dilution or mechanical disruption. For example, the solvating system may with

appropriate care be applied as a pressurized spray to dislodge the bacterial biofilm, bacteria and other foreign body buildup at the treatment site. This may be accompanied by breakdown of the biofilm EPS matrix through calcium ion sequestering by the metal ion sequestering agent, and by solvation of the resulting breakdown fragments (e.g., mannuronic and guluronic acids) into aqueous solution so as to facilitate their ultimate removal using aspiration, lavage or other removal techniques performed via the myringotomy or through the Eustachian tube, nose or mouth. It may be desirable to inject sufficient solvating system into the treatment area to displace any pus or other material that may be present, allowing excess material to overflow from the treatment area until the color of the excess material no longer changes. The solvating system may be left in place until it can drain away or is otherwise eliminated or resorbed, or the solvating system may be allowed to stand for a suitable time (e.g., a few minutes, a few hours or longer) and then may be rinsed away using saline or another suitable liquid. The solvating system preferably is applied directly to the treatment site, as such direct application may promote faster biofilm breakup. For example, for procedures performed in the middle or inner ear the solvating solution preferably is applied directly into the middle or inner ear region rather than merely being applied to the ear canal and allowed to transport across the tympanic membrane. Application of the solvating system and removal of dislodged or disrupted biofilm and bacteria may also be repeated as desired to ensure thorough removal of the offending organisms.

[0043] The solvating system may desirably be used as a part of a multi-step treatment regimen which disrupts the bacterial biofilm and discourages its return. For example, a series of steps that may be broadly classified as Cleansing/Disrupting, Killing, Protecting/Coating, Aerating, and Healing may be carried out. The Cleansing/Disrupting step may be carried out by administering the solvating system as described above. The Killing step may be carried out by applying a suitable antimicrobial agent to the treatment site. This may for example be accomplished by including an antimicrobial agent in the solvating system or by separately applying such an agent intra operatively or post operatively (e.g., topically, orally or systemically). The Protecting/Coating step may be carried out by coating at least part of the thus-treated tissue with a protective sealant layer. The sealant may provide a variety of benefits such as discouraging or preventing

recolonization of the tissue surface with bacteria and new biofilm-forming colonies; reducing inflammation; improving wound healing or allowing for the recovery of the body's natural innate immune response. The sealant may include one or more antimicrobial agents to further attack any bacterial biofilm, biofilm fragments or bacteria remaining following the Cleansing/Disrupting step described above. Further details regarding a preferred sealant may be found in the above-mentioned copending application Serial No. (attorney docket no. 151-P-28168WO01). The Aerating step may be carried out by preserving or forming a suitable opening or openings (e.g., a slit in the tympanic membrane, or an opening in occluded or partially occluded nasal passages, sinuses or sinus ostia) and leaving it or them open for a period of time sufficient to allow aeration of the treatment site. The time period may be affected by the nature of the opening(s) and for ear treatments by whether or not a tympanostomy tube is installed. For example, if a slit has been formed in the tympanic membrane and a tube is not placed in the opening then the slit may remain open for a few days and heal over, thereby closing the ear space naturally. The Healing step may be carried out by allowing the cleansed, protected and sealed tissue surface to undergo a return to a normal state, e.g., through one or more healing mechanisms such as modulation of an inflammatory response, phagocytosis, mucosal remodeling, reciliation or full or partial restoration of normal hearing or balance.

[0044] The invention is further illustrated in the following non-limiting examples.

Example 1

[0045] Bacterial isolates of *S. aureus* and *P. aeruginosa* bacteria were recovered from the sinuses of patients with sinus disorders. Patients with cystic fibrosis or an underlying immunosuppressive disease (HIV infection, insulin-dependent diabetes mellitus, or renal disease) and patients who had taken antibiotics or oral prednisone in the previous month were excluded. All patients had refractory sinusitis, that is, persistent symptoms resistant to medical therapy despite having undergone technically successful functional endoscopic sinus surgery (FESS) for refractory chronic rhinosinusitis (CRS) with or without nasal polyposis. The occurrence of CRS was diagnosed in accordance with the 2003 American Academy of Otolaryngology–Head and Neck Surgery (AAO-HNS) guidelines set out in Benninger et al., "Adult chronic rhinosinusitis: Definitions, diagnosis, epidemiology, and

pathophysiology", Otolaryngol Head Neck Surg 129(3 suppl):S1-S32 (2003). The selected patients had been refractory to medical therapy for more than 12 months before sample collection, and the failure of FESS was judged not to be associated with technical factors such as obstructive synechiae, frontal sinus obstruction, or a retained uncinate process. Samples were collected consecutively until 10 specimens each of *S. aureus* and *P. aeruginosa* were obtained using direct endoscopic guidance and the procedure described by Nadel et al., "Endoscopically guided cultures in chronic sinusitis", Am J Rhinol 12:233-241 (1998). Briefly, a topical anesthetic agent was administered, the nasal ala retracted, and an endoscope used to visualize the middle meatus and sinus cavities. A thin, flexible calcium alginate swab (STARSWAB II™ Collection and Transport System, Starplex Scientific, Etobicoke, Ontario) was inserted and directed to the site with the most purulence. If no purulence was observed, the surface of the maxillary sinus was swabbed for 15 seconds. Care was taken to avoid contact with the lateral nasal wall or nasal vestibule. Samples were plated and incubated using standard procedures. Bacteria were identified using a VITEK 2™ system (Biomérieux, Durham, NC). Crystal violet staining to confirm the presence of biofilms was performed according to the method described by Stepanovic et al., "A modified microtiter-plate test for quantification of staphylococcal biofilm formation", J Microbiol Methods 40:175-179 (2000). For incubation and culture, previously frozen strains were inoculated on trypticase soy agar (TSA) with 0.5% sheep blood. After 24 hours, one to four colonies per strain were cultured on TSA. Cultures were incubated at 37°C for 24 hours to condition them to a trypticase soy broth (TSB)–TSA medium and ensure noncontamination. Colonies grown on TSA solid medium were then amplified in 5 mL of TSB medium with 0.5% glucose according to the method described by Gotz, "Staphylococcus and biofilms", Mol Microbiol 43:1367-1378 (2002) and incubated at 37°C for at least 24 hours.

[0046] A drip-flow reactor (DFR) was used to determine the effectiveness of various test solutions delivered to *S aureus* and *P aeruginosa* biofilms on hydroxyapatite (HA)-coated microscope slides for removing these bacterial biofilms with and without hydrodynamic force. The slides in the DFR are tipped at 10° from the horizontal, thereby modeling a low shear environment. The DFR was housed in an incubator at 37°C under aerobic conditions. Approximately 20 minutes before bacterial inoculation, sterile

medium (10% TSB for *S aureus*; 1% TSB for *P aeruginosa*) was dripped on the slides in the DFR and allowed to collect over the slides to form a conditioning layer. The slides were then inoculated with 1 mL of a culture of either *S aureus* or *P aeruginosa*. The DFR was tilted so that the slides would be horizontal for 4 hours to allow bacterial attachment to the substrate. Subsequently, the DFR was set so that the slides were once again at a 10° angle, with sterile medium dripping on the slides at a rate of 10 mL per hour. After 3 days, biofilm-removal experiments were performed. Two methods were used to treat the biofilms formed by each bacterial species. The first application method involved a static treatment in the DFR, with a solvating agent (referred to as CAZS) being dripped onto the biofilms. The CAZS solvating agent contained deionized water, 25 g/L (corresponding to 0.13 M) citric acid, 5.35 g/L (corresponding to 0.02 M) caprylyl sulfobetaine zwitterionic surfactant ($CH_3(CH_2)_9N^+(CH_3)_2CH_2CH_2CH_2SO_3^-$, CAS 15163-36-7) and sufficient sodium citrate (about 240 g/L) to buffer the system to pH 5.4. The second application method involved delivery of saline or delivery of CAZS outside the DFR, using a pressurized jet lavage to apply a hydrodynamic shearing force to the biofilm. For all treatments, preliminary runs were done to ensure that variations among slides were within acceptable limits. In addition, multiple plates of both bacterial species were produced to determine the within-run and run-to-run variations. A control slide was made for each DFR run. Three runs were evaluated for each treatment of each type of bacteria.

[0047] For static treatment, flow to the DFR was halted, the DFR was placed in a horizontal position, and the cover was removed. A 25 mL portion of CAZS was applied to one slide. Control slides were not treated with CAZS. After 10 minutes, the slides were rinsed with saline (25 mL). The DFR was then disconnected from the inflow tube, and each slide was removed under a laminar flow hood and placed in a sterile 50-mL tube. After another saline rinse (2 mL), the surface of the slide was scraped repeatedly, and the scrapings and saline were collected in the tube. The tube was vortexed for 10 seconds, sonicated for 2 minutes, and vortexed again for 10 seconds to disperse the bacteria into suspension. The suspensions were then serially diluted and 100-μL aliquots applied to three plates containing TSA and incubated at 37°C for 24 hours. Colony-forming units (CFUs) were counted manually, and the number of CFUs per square centimeter was

calculated. The resulting plate counts were log (10) transformed and expressed as the mean (± SD) value derived from plate counts from two DFR runs of three slides each.

[0048] For hydrodynamic treatment, the slides were removed from the DFR and placed in a glove box. The slides were placed in a holder and sprayed for approximately 20 seconds with about 150 mL of either saline or CAZS using a device that provided pressurized jet lavage. The spraying was done with both a side-to-side and an up-and-down sweeping motion so that all areas were sprayed twice, once in each axis. The slides were then placed in sterile 50-mL tubes, rinsed, scraped, dispersed, incubated and evaluated as described above.

[0049] The mean (± SD) percent reduction from control values in the quantity of *S. aureus* and *P. aeruginosa* bacteria (*viz.*, the number of CFUs on each plate) after each treatment was calculated and the results assessed using two-sample *t* tests (MINITAB™ version 14, Minitab, State College, PA). A *P* value less than 0.05 was considered to represent a significant difference from the control value. The results are shown below in Table 1, expressed as the mean (± SD) number of colony-forming units per centimeter (log) derived from three plates assessed twice:

Table 1

Bacterial Plate Log Counts According to Type of Treatment

Treatment	*Staphylococcus aureus*	*Pseudomonas aeruginosa*
None (Control)	8.7 ± 0.4	9.2 ± 0.2
Static CAZS delivery	6.2 ± 0.3	6.3 ± 1.3
Hydrodynamic saline delivery	6.4 ± 0.2	6.9 ± 0.1
Hydrodynamic CAZS delivery	4.8 ± 0.3	4.0 ± 0.5

[0050] The results in Table 1 show that significant bacterial biofilm removal was obtained. Before treatment, ample biofilms formed in the DFR cultures of both *S. aureus* and *P. aeruginosa*, with CFU counts for these Controls ranging from 7.8 to 9.5 log/cm^2.

Static administration of CAZS resulted in a 2.5 log reduction (5.11×10^8 to 1.65×10^6; $P = 0.001$) in the number of *S. aureus* CFUs and a 2.9 log reduction (1.69×10^9 to 1.91×10^6; $P = 0.002$) in the number of *P. aeruginosa* CFUs. Mechanical disruption using hydrodynamic saline delivery alone decreased the number of *S. aureus* CFUs by 2.3 log units (5.11×10^8 to 2.38×10^6; $P = 0.001$) and the number of *P. aeruginosa* CFUs by 2.4 log units (1.69×10^9 to 7.31×10^6; $P = 0.001$). However, mechanical disruption using hydrodynamic CAZS decreased the *S. aureus* CFU count by 3.9 log units (5.11×10^8 to 6.37×10^4; $P = 0.001$) and the *P. aeruginosa* CFU count by 5.2 log units (1.69×10^9 to 1.04×10^4; $P = 0.001$).

[0051] Confocal scanning laser microscopy (CSLM) was performed on three slides (for each treatment and bacteria species) not subjected to plate counts to allow imaging of the biofilm architecture in control and treated samples. The slides were stained for CSLM using a BACLIGHT™ Live/Dead kit (Molecular Probes, Invitrogen, Carlsbad, CA) containing two nucleic acid stains (SYTO 9, which detects living cells by fluorescing green, and propidium iodide, which detects dead cells by fluorescing red). After staining, the slides were examined using CSLM at a 630X magnification using a LEICA™ SP2 acoustic-optical beam splitter with a 2-photon MAI TAI™ attachment (Leica Microsystems, Bannockburn, IL) and fluorescence excitation and detection in both the green and red spectra. Each slide area was divided into 10 equally sized segments. A microscopic field was selected at random from each segment, and images were obtained at 1-μm intervals from the top of the biofilm to the substrate, thereby creating an image stack for each location. The CSLM analysis revealed that a thick biofilm carpeted the Control slides. Hydrodynamic treatment with saline and static treatment with CAZS decreased the amount of biofilm coverage markedly and reduced the organization of the remaining biofilm. Hydrodynamic treatment with CAZS produced a greater reduction both in biofilm coverage and in the amount of order in the biofilm community. The results corresponded generally to the plate count assessments with respect to the relative reductions in the amount of biofilm achieved with each treatment.

[0052] Of the three treatments investigated, power irrigation using CAZS and a pressurized jet lavage was the most effective in disrupting the bacterial biofilms. Power irrigation using saline had appreciable biofilm-reducing effects. However, the presence of

a surfactant and citric acid in the irrigation solution significantly enhanced the reduction in CFU count in both *S. aureus* and *P. aeruginosa* biofilms. Large, statistically significant reductions occurred, with the mean decreases in bacterial plate counts being 3.9 and 5.2 log (a reduction of 10,000 to 100,000 times), respectively, for *S. aureus* and *P. aeruginosa* biofilms. A decrease of this magnitude *in vitro* indicates that an appropriate *in vivo* treatment in the middle or inner ear, nasal or sinus cavities or oral or pharyngeal tissues should effectively disrupt bacterial biofilms found there. Any remaining low level of persistent bacterial infection might be dealt with by host defenses or a topically or orally administered antimicrobial agent, and by application of a sealant as described above.

Example 2

[0053] The CAZS solvating system employed in Example 1 was modified by replacing some of the water with gallium nitrate so that the modified system contained 25% gallium nitrate. A Control solution containing 25% gallium nitrate in deionized water was also prepared. When evaluated using the static treatment technique of Example 1, administration of the gallium nitrate Control solution resulted in a 3.4 log reduction (average of 4 runs) in the number of *S. aureus* CFUs and a 4.1 log reduction (average of 3 runs) in the number of *P. aeruginosa* CFUs. Static administration of the solution containing CAZS and gallium nitrate resulted in a 5.2 log reduction (average of 2 runs) in the number of *S. aureus* CFUs and a 5.5 log reduction (average of 2 runs) in the number of *P. aeruginosa* CFUs.

[0054] Although specific embodiments have been illustrated and described herein for purposes of description of the preferred embodiments, it will be appreciated by those of ordinary skill in the art that a wide variety of alternate or equivalent implementations calculated to achieve the same purposes may be substituted for the specific embodiments shown and described without departing from the scope of the present invention. This application is intended to cover any adaptations or variations of the preferred embodiments discussed herein. Therefore, it is manifestly intended that this invention be limited only by the claims and the equivalents thereof.

We claim:

1. The use of a metal ion sequestering agent and greater than 0.2 wt. % of a surfactant which can detach, remove or otherwise disrupt at least a part of a biofilm attached or adhered to at least a portion of the middle or inner ear, to a surface within a nasal or sinus cavity, or to oral or pharyngeal tissue, to manufacture a medicament for the treatment of bacterial ear, nose or throat conditions.

2. The use of (a) a metal ion sequestering agent, (b) a zwitterionic surfactant which can detach, remove or otherwise disrupt at least a part of a biofilm attached or adhered to at least a portion of the middle or inner ear, to a surface within a nasal or sinus cavity, or to oral or pharyngeal tissue, and (c) sufficient buffer to provide a pH greater than 5, to manufacture a medicament for the treatment of bacterial ear, nose or throat conditions.

3. A method for treating bacterial ear, nose or throat conditions, which method comprises:

a) applying a solvating system comprising a metal ion sequestering agent and greater than 0.2 wt. % surfactant to a bacterial biofilm attached or adhered to at least a portion of the middle or inner ear, to a surface within a nasal or sinus cavity, or to oral or pharyngeal tissue, and

b) detaching, removing or otherwise disrupting at least a part of the biofilm.

4. A method for treating bacterial ear, nose or throat conditions, which method comprises:

a) applying a solvating system comprising a metal ion sequestering agent, a zwitterionic surfactant, and sufficient buffer so that the solvating system has a pH greater than 5 to a bacterial biofilm attached or adhered to at least a portion of the middle or inner ear, to a surface within a nasal or sinus cavity, or to oral or pharyngeal tissue, and

b) detaching, removing or otherwise disrupting at least a part of the biofilm.

5. A method according to claim 3 or claim 4 wherein the solvating system is applied to at least a portion of the middle ear.

6. A method according to claim 3 or claim 4 wherein the solvating system is applied to a nasal or sinus cavity

7. A method according to any of claims 3 to 6 comprising applying the solvating system by spraying, lavage, misting, mopping, wicking or dripping and further comprising removing the solvating system by flushing, rinsing, draining or absorption.

8. A use according to claim 1 or claim 2 or a method according to claim 3 or claim 4 wherein the metal ion sequestering agent comprises a mild acid whose acidity is sufficient to sequester one or more metal ions in the bacteria biofilm but which is not so acidic so as to harm the middle or inner ear.

9. A use according to claim 1 or claim 2 or a method according to claim 3 or claim 4 wherein the metal ion sequestering agent comprises a sequestering agent for sodium, calcium or iron.

10. A use according to claim 1 or claim 2 or a method according to claim 3 or claim 4 wherein the metal ion sequestering agent comprises a carboxylic acid, diacid, triacid or mixture thereof.

11. A use according to claim 1 or claim 2 or a method according to claim 3 or claim 4 wherein the metal ion sequestering agent comprises formic acid, acetic acid, chloroacetic acid, dichloroacetic acid, oxalic acid, oxamic acid, glycolic acid, lactic acid, pyruvic acid, aspartic acid, fumaric acid, maleic acid, succinic acid, iminodiacetic acid, glutaric acid, 2-ketoglutaric acid, glutamic acid, adipic acid, glucuronic acid, mucic acid, nitrilotriacetic acid, salicylic acid, ketopimelic acid, benzoic acid, mandelic acid, chloromandelic acid, phenylacetic acid, phthalic acid, boric acid or mixture thereof.

12. A use according to claim 1 or claim 2 or a method according to claim 3 or claim 4 wherein the metal ion sequestering agent comprises citric acid.

13. A use according to claim 1 or claim 2 or a method according to claim 3 or claim 4 wherein the metal ion sequestering agent is present at a concentration of about 0.01 to about 0.5 M.

14. A use according to claim 1 or claim 2 or a method according to claim 3 or claim 4

15. A use according to claim 1 or claim 2 or a method according to claim 3 or claim 4 wherein the solvating system has a pH less than 8.5.

16. A use according to claim 1 or claim 2 or a method according to claim 3 or claim 4 wherein the solvating system further comprises an antimicrobial agent.

17. A solvating system for disrupting bacterial biofilms on tissue, the composition comprising a metal ion sequestering agent, greater than 0.2 wt. % surfactant, and an antimicrobial agent.

18. A solvating system for disrupting bacterial biofilms on tissue, the composition comprising a metal ion sequestering agent, a zwitterionic surfactant, and sufficient buffer so that the solvating system has a pH greater than 5.

19. A solvating system according to claim 17 or claim 18 wherein the metal ion sequestering agent comprises a mild acid whose acidity is sufficient to sequester one or more metal ions in the bacteria biofilm but which is not so acidic so as to harm the middle or inner ear portion to which the solvating system is applied.

20. A solvating system according to claim 19 wherein the metal ion sequestering agent comprises a sequestering agent for sodium, calcium or iron.

21. A solvating system according to claim 19 wherein the metal ion sequestering agent comprises a carboxylic acid, diacid, triacid or mixture thereof.

22. A solvating system according to claim 21 wherein the metal ion sequestering agent comprises formic acid, acetic acid, chloroacetic acid, dichloroacetic acid, oxalic acid, oxamic acid, glycolic acid, lactic acid, pyruvic acid, aspartic acid, fumaric acid, maleic acid, succinic acid, iminodiacetic acid, glutaric acid, 2-ketoglutaric acid, glutamic acid, adipic acid, glucuronic acid, mucic acid, nitrilotriacetic acid, salicylic acid, ketopimelic acid, benzoic acid, mandelic acid, chloromandelic acid, phenylacetic acid, phthalic acid, boric acid or mixture thereof.

23. A solvating system according to claim 21 wherein the metal ion sequestering agent comprises citric acid.

24. A solvating system according to claim 17 or claim 18 wherein the surfactant comprises an alkyl sulfate, alkyl sulfonate or aryl sulfonate or mixture thereof.

25. A solvating system according to claim 17 or claim 18 comprising an antimicrobial agent comprising a chitosan salt, cubic-phase lipid, gallium-containing compound, carboxylic ester, sulfonic acid, active halogen compound, active oxygen compound, organic peroxide, ozone, singlet oxygen generator, phenolic derivative or quaternary ammonium compound.

26. A solvating system according to claim 25 wherein the antimicrobial agent comprises gallium acetoacetonate, gallium bromide, gallium chloride, gallium fluoride, gallium iodide, gallium maltolate, gallium nitrate, gallium nitride, gallium percolate, gallium phosphite, gallium sulfate or mixture thereof.

27. A solvating system according to claim 17 or claim 18 wherein the metal ion sequestering agent is present at a concentration of about 0.01 to about 0.5 M.

28. A solvating system according to claim 17 or claim 18 wherein the surfactant is about 0.5% to about 25% of the solvating system.

29. A solvating system according to claim 17 or claim 18 comprising buffer and having a pH greater than 5 and less than 8.5.

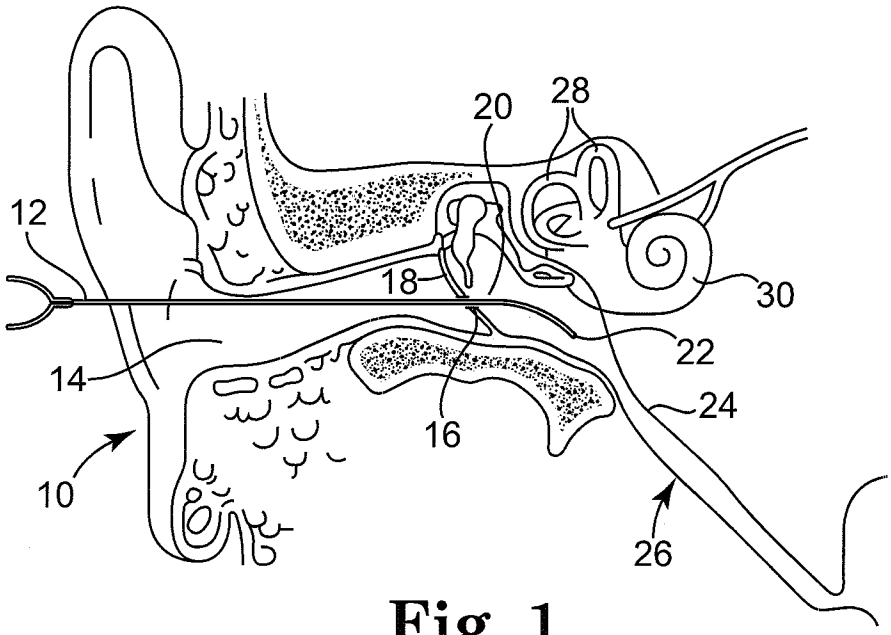


Fig. 1

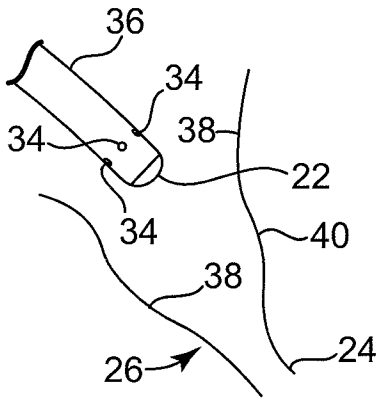


Fig. 2

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2007/068477

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61P27/16 A61P31/04 A61K31/10 A61K31/19 A61K33/22
A61K45/06 A61K33/24 A61K33/40

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2006/029255 A (3M INNOVATIVE PROPERTIES CO) 16 March 2006 (2006-03-16) page 4, line 9 - page 5, line 11; claims 1-5,10,16-18,21,25-27,55-60,66-69 page 31, lines 22-28 page 34, line 26 - page 35, line 11 page 41, lines 4-10 page 49, lines 24-26 page 50, lines 30-32 page 68, lines 8-14 page 79, lines 23-29; example 2	1-25, 27-29
X	EP 0 530 861 A2 (KABARA JON JOSEPH [US]) 10 March 1993 (1993-03-10) page 7, lines 12-20; claims 1,8-10 page 2, lines 3-15	1-23,25, 27-29

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 September 2007	25/09/2007

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Kanbier, Titia

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2007/068477

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2006/026083 A (QPHARMA LLC) 9 March 2006 (2006-03-09) pages 8-10; claims 1,8-11,18,19 page 11, lines 23,24	1-4, 6-23,25, 28,29
X	WO 2005/015996 A (ECOLAB INC) 24 February 2005 (2005-02-24) pages 41-42; claims 1,12-17 pages 27-28; tables 1-4 page 17, paragraph 2	1-4,8-23
X	WO 01/57174 A (RECKITT BENCKISER INC) 9 August 2001 (2001-08-09) page 19, lines 1-5; claims 1,3,4,11-13 page 25, lines 14-19 page 4, lines 8-19	1-4, 17-24, 27-29
X	US 2006/035808 A1 (AHMED FAHIM U [US] ET AL) 16 February 2006 (2006-02-16) paragraphs [0123] - [0125]; claims 1,6,7,11,12,21,22,45-52 pages 15-16, paragraph 60; table 5	1-6, 17-25
X	WO 2006/007371 A (JOHNSON & SON INC S C) 19 January 2006 (2006-01-19) paragraphs [0030] - [0032]; claims 1,6-8,11	17-25,29
A	WO 03/061579 A (UNIV EMORY) 31 July 2003 (2003-07-31) page 8, line 31 - page 10, line 10 page 29, lines 1-3; claims 1,5,11,15,19	1-4, 16-18, 25,26,29
A	WO 98/09622 A (UNIV IOWA RES FOUND) 12 March 1998 (1998-03-12) page 15, lines 15-19; example 5 page 8, lines 10-16; claims 1,19,29	25,26
A	US 2005/042240 A1 (UTTERBERG D S) 24 February 2005 (2005-02-24) claims 1,2,10,15	16,17,25
A	EP 0 933 081 A1 (AGIS IND 1983 LTD) 4 August 1999 (1999-08-04) page 2, paragraph 8-11; claims 1,2,8-11	1-7, 16-18

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2007/068477

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 3-16 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2007/068477

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